

# **Brazilian Journal of Animal and Environmental Research**

## **Evaluation of balneability of waterfalls by microbiological standards**

### **Avaliação da balneabilidade de cachoeiras por padrões microbiológicos**

Recebimento dos originais: 01/10/2019

Aceitação para publicação: 29/11/2019

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#### **ABSTRACT**

The objective of our work was to characterize, as to bathing, two waterfalls that are much visited in the city of Poços de Caldas - MG, the Pedra Balão waterfall and Lua de Mel waterfall. We performed the characterization of the waterfalls according to CONAMA 274/2000 standards and the analysis methodology was by membrane filtration (FM) technique and incubation in a selective chromogenic medium, according to Standard Methods for the Examination of Water and Wastewater (APHA, 1995). We obtained satisfactory bathing results for the waterfalls. The results presented, allow a better control

by the municipal agencies regarding the use of these waterfalls, in order to reduce the risk of diseases for residents and tourists visiting these places.

**Keywords:** Balneability, Microbiology, Water resources.

## RESUMO

O presente trabalho teve por objetivo caracterizar quanto à balneabilidade, duas cachoeiras muito visitadas no município de Poços de Caldas - MG, a cachoeira da Pedra Balão e cachoeira Lua de Mel. A caracterização das cachoeiras foi realizada conforme as normas da resolução CONAMA 274/2000 e a metodologia de análise se deu por meio da técnica de filtração por membrana (FM) e incubação num meio seletivo cromogênico, de acordo com *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995). Foram obtidos resultados de balneabilidade satisfatórios para as cachoeiras. Os resultados apresentados, possibilitam um melhor controle por parte dos órgãos municipais quanto ao uso dessas cachoeiras, a fim de diminuir o risco de doenças para moradores e turistas que visitem esses locais.

**Palavras-chave:** Balneabilidade, Microbiologia, Recursos hídricos.

## 1 INTRODUCTION

Poços de Caldas is a municipality visited by tourists from different parts of the country, for its famous hydromineral and hot springs. Having high rainfall rates that according to PMPC (2017) reaches an annual average of 1745 mm and with approximately 190 days of rain, which enables a rich hydrological diversity, such as waterfalls, dams and rivers. The waters intended for the treatment system are monitored monthly by the Municipal Department of Water and Sewer - DMAE, however the waters intended for leisure, such as waterfalls and dams still need to be monitored for their bathing, given the few existing studies in the municipality.

According to Berg et al. (2013), balneability is the ability of a place to enable bathing and sports activities in its waters, ie, the quality of water intended for primary contact recreation. For recreation purposes, some specific water quality requirements are necessary, considering the health risk posed by direct and prolonged exposure to pathogenic organisms present in contaminated water bodies (LOPES et al., 2013).

The National Environmental Council Resolution - CONAMA 274/2000 classifies the conditions of bathing, evaluating them in categories defined according to the amount of thermotolerant coliforms or *Escherichia coli*. To make this classification the results of the last five weeks of collection are used.

Importantly, these microorganisms are chosen for this type of analysis because they are related to fecal contamination. Total coliforms can be found in a free-living environment, however *Escherichia coli* is an exclusive indicator of fecal contamination, and for this reason are adopted as control

parameters, besides the fact that laboratory tests for identification of this species are rapid, with international standardization, and accepted for routine use (CONAMA, 2000; BORGES et al., 2015).

The aim of our work was to evaluate the balneability of two waterfalls in the Poços de Caldas region, known as Pedra Balão waterfall and Lua de Mel waterfall, by microbiological standards.

## **2 METHODOLOGY**

### **a. Study area characterization**

The waterfalls under study are located in the city of Poços de Caldas, presenting distinct environmental characteristics. Pedra Balão Waterfall has native vegetation in the surrounding area in a better state of conservation than the Lua de Mel Waterfall, which is bordered by a short vegetation cover and near Avenida João Pinheiro.

The selection of the study site was based on the criteria of anthropogenic recreational influence, since the sampling places are used as a source of recreation by the population that visits these sights.

#### *i. Pedra Balloon Waterfall*

The first sampling point is located near a tourist center called Pedra Balão, which requires a trail of approximately 30 minutes to access the waterfall. The waterfall is hidden among the closed forest, and has dimensions of 8m high, and a well with depth of 4m by 3m wide, being visited mostly on weekends and holidays.

The access trail is in a state of erosion with the exposed soil, dragging sandy and organic sediments derived from the remains of vegetation covering the main trail, and the vegetation that accompanies the trail consists of freshly cut eucalyptus. In the regions closest to the waterfall you can see typical fragments of Atlantic forest in good condition, such as ferns, embaúba, jequitibá among other species typical of this biome.

The region is inhabited by small native mammals such as coatis, rodents and capuchin monkeys, as well as domestic animals such as dogs, which eventually appear in the body of water to feed or drink water.

#### *ii. Honeymoon Waterfall*

The second sampling point is the Honeymoon Waterfall, located on the Av. João Pinheiro highway, which has a ticket for a tourist restaurant, which offers access to the waterfall, it is connected with the Ribeirão das Antas, which is a tributary of the Paraná River. (ALBERTI & PEREIRA, 2008).

This is a region close to residential developments and Avenida João Pinheiro, with intense and very busy traffic. Solid wastes such as plastics are found near the easily accessible water body and are commonly used by swimmers.

The riparian forest that surrounds the analyzed point is small, around 10 to 15m, with oscillations along the course of the main river, presenting, as in Cachoeira da Pedra Balão, fragments of Atlantic forest, but in a smaller state of conservation.

### **b. Sample Collection**

The experimental procedure consisted of a total of 10 samples, collected at two points in the city of Poços de Caldas / MG. We collected the samples weekly starting on October 04, 2017, for a period of 5 weeks, at 09:30. on every day of collection for the first point, at Cachoeira da Pedra Balão, and at 11:00 at the second point, at the Honeymoon Waterfall, making sure that there were no sediments entering the bottle and no contact with rocks. or vegetal fragments, aiming at the necessary care to avoid the contamination of the samples. We also record the weather and visual characteristics of the water.

### **c. Analysis Methods**

After we collect the samples at the two points already mentioned, according to standard methods (SALUD, 1998), we store the samples in the ice-cold transport box and then take them to the microbiology lab, where we carry out the microbiological studies. using the membrane filtration technique (FM) and incubation in a selective medium (SALUD, 1998) (MERCK, 2014).

In the laboratory we performed  $10^{-2}$  and  $10^{-4}$  dilutions for each sample, placed 100 ml of pure samples and their dilutions in different beakers so that they could be vacuum filtered through a 0.45  $\mu\text{m}$  pore cellulose membrane Unifil brand.

We did the cultivation in selective differential Chromocult from Merck brand previously made according to the manufacturer's indications (MERCK, 2014), and we counted CFU colonies 24 hours after incubation at 37 ° C.

### **d. Analysis of results according to CONAMA resolution 274/2000**

Microbiological standards for water quality vary by country, this determination is made by environmental agencies that are responsible for setting the required parameters according to regional conditions. Resolution 274 of November 29, 2000, establishes the classification for the quality of freshwater, brackish and saline intended for bathing, in that same resolution in Article 2, is determining

the classification of waters according to the conditions evaluated, being divided into categories. (CONAMA, 2000):

*“Art. 2nd Fresh, brackish and saline waters intended for bathing (primary contact recreation) will be evaluated in their own and improper categories.*

*Paragraph 1. The waters considered proper may be subdivided into the following categories:*

*(a) Excellent: when at most 80% or more of a set of samples taken in each of the previous five weeks taken from the same site there are a maximum of 250 (thermotolerant) faecal coliforms or 200 Escherichiacoli or 25 enterococci per 100 milliliters. ;*

*(b) Very Good: when at most 80% or more of a set of samples taken in each of the previous five weeks taken at the same site there is a maximum of 500 (thermotolerant) faecal coliforms or 400 Escherichia coli or 50 enterococci per 100 milliliters;*

*(c) Satisfactory: when in 80% or more of a set of samples taken in each of the previous five weeks taken from the same site, there are at most 1,000 faecal coliforms (thermotolerants) or 800 Escherichia coli or 100 enterococci per 100 milliliters.”.*

## 3 RESULTS AND DISCUSSION

We put our environmental observations of the sample collection days in Table 1.

	Features	Pedra Balão Walterfall	Lua de Mel Walterfall
	Date: 04/10/17	04.10.2017	04.10.2017
1ª week	Atmosphere conditions:	Sunny	Sunny
	Water visual characteristics:	Clear water	Clear water
	Date: 10.10.2017	10.10.2017	10.10.2017
2ª week	Atmosphere conditions:	Cloudy	Cloudy
	Water visual characteristics:	Águas turvas	Slightly blurred
	Date: 18.10.2017	18.10.2017	18.10.2017
3ª week	Atmosphere conditions:	Sunny	Sunny
	Water visual characteristics:	Clear water	Clear water
	Date: 25.10.2017	25.10.2017	25.10.2017
4ª week	Atmosphere conditions:	Cloudy	Cloudy
	Water visual characteristics:	Slightly blurred	Slightly blurred
	Date: 25.11.2017	25.11.2017	25.11.2017
5ª week	Atmosphere conditions:	Cloudy	Sunny
	Water visual characteristics:	Clear water	Clear water

Table 1. Meteorological conditions and visual characteristics of the waters during the collections.

From the microbiological analyzes, we performed the counting of the studied microorganisms (total coliforms, *Escherichia coli*), which we cultivated in our own environment, called Chromocult. In addition, we analyzed the microbial growth in the pure sample and in two dilutions for better reading. We divided the samples from the collected points into two: Sample A and Sample B, referring to the first and second collection points, respectively, and can be seen in Figure 1 showing the growth results of these microorganisms in the specific culture medium.

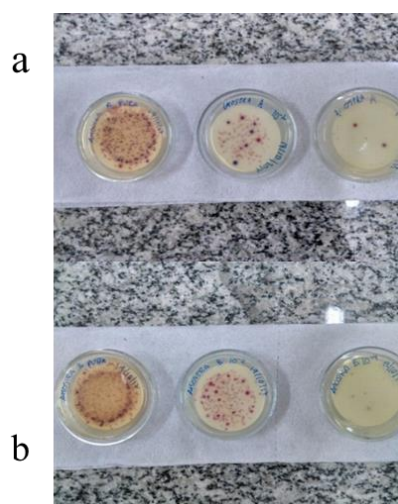


Figure 1. (a) Sample collected on 10/19/2017 at point A, contains pure sample, 10<sup>-2</sup> diluted sample and 10<sup>-4</sup> diluted sample, respectively. (b) Sample collected on 10/19/2017 at point B contains pure sample, 10<sup>-2</sup> diluted sample and 10<sup>-4</sup> diluted sample.

The other samples follow the same pattern as those shown in Figure 1, varying the number of colonies as represented by Figures 2 and 3 expressing the results from the microorganism count during the five weeks at both collection sites.

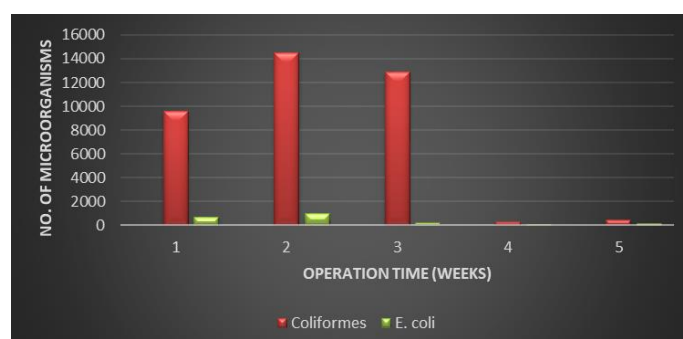


Figure 2. Microorganism count in the samples of Pedra Balão waterfall.

From Figure 2, it could be inferred that sample A is in its own category and is satisfactory, since 80% or more of its sample set in five weeks of analysis, collected at the same place, has at most 800 *Escherichia coli* according to CONAMA Resolution 274/2000.

In addition, we swam that in the second week, when the samples were collected at the first point (Sample A - Cachoeira da Pedra Balão), the water was turbid, because during two days before the collection there were accentuated precipitation events in this region, which may have promoted the mixing of plant and faecal organic material from native fauna that may be associated with higher coliform and *Escherichia coli* values on that day.



Figure 3. Microorganism count in the samples of the Honeymoon Waterfall.

Sample B presents 100% of *Escherichia coli* samples in up to 800 colonies, which according to the same resolution is classified as suitable for bathing in the satisfactory category.

Thus, from the results found, we find that Cachoeira da Pedra Balão falls as suitable for bathing, in the satisfactory category, as 80% of the samples meet the prerequisite of this category, while the Waterfall Honeymoon meets the requirements minimum bathing in 100% of the samples, thus fitting as suitable for bathing also in the satisfactory category according to CONAMA Resolution 274/2000.

#### 4 CONCLUSION

Comparing the results, we realize that the Cachoeira da Pedra Balão presented an average of 9,600 total coliforms and 194 *E. coli*, while Lua de Mel waterfall presented an average of 16,482 total coliforms and 700 *E. coli*, according to the data obtained in the 5 weeks of analysis. Therefore, through this work it was possible to identify in which legal framework the two studied waterfalls are, so that, according to the results obtained we find that both waterfalls are suitable for bathing in the same category, satisfactory.



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